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THE SKATE AS A SUBJECT FOR CLASSES IN COMPARATIVE ANATOMY; INJECTION METHODS.

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THE selection and obtaining of material for the laboratory work of classes in zoölogy, and the best methods of treating that material, are matters of considerable importance to those having such work in charge. My use of the skate for several years past has impressed me with the belief that it is, in some respects, peculiarly adapted for use in the laboratory work of classes in the comparative anatomy of vertebrates. If I am not mistaken, the advantages of the skate for this purpose and the practicability of using it are not always fully realized, especially by teachers who are situated at some distance from the sea. In view of this fact, I think it worth while to call attention to certain points wherein the skate seems to me to be a superior subject for laboratory work. In addition, I shall consider methods of injecting the circulatory system of this fish.

If a student in comparative anatomy is to study a fish as a representative of the class, obviously it should be one which exhibits as nearly as possible primitive vertebrate conditions, — therefore an elasmobranch. If an amphibian is to follow the fish, it should, of course, be one of the tailed amphibians rather than the highly specialized frog. The elasmobranch, in its general anatomy, is much more directly and closely comparable with the urodele than is any teleost. To start a student in vertebrate anatomy on the dissection of a teleost and to follow that with the frog, as a representative of Amphibia, is as poor a program as could be arranged, if it is the intention that he should

see for himself something of the main course in the evolution of the structure of higher vertebrates. The study of types situated near the main line of vertebrate descent is to be preferred to the study of extremes of specialization. It seems to me to be questionable wisdom to let enthusiasm for local fauna lead to the selection of a teleost, for class study, provided that marine material is obtainable, and provided further that only one representative of Pisces can be studied.

Among the elasmobranchs, the sharks depart less conspicuously from primitive conditions than the rays and therefore, theoretically, the dogfishes are to be preferred to the skates for general laboratory purposes. The dorso-ventral flattening of the skate, however, gives it a certain superiority as a subject for dissection. As a result of the flattening, most of the organs of the animal are much more easily accessible than they are in the dogfish. This advantage is of particular importance when it comes to the injection and dissection of the blood-vessels.

The abdominal cavity of the skate is completely exposed with a minimum of cutting. This cavity is a broad and very shallow one so that, its ventral wall having been removed, a slight displacing of viscera brings all its organs immediately into view and renders its chief blood-vessels very easy of access.

It is a consideration of no small moment that the skate, during injecting and dissecting operations, lies in the position most favorable for work — that is, flat on its back — without any special device for holding it there, while it is not always easy to support a dogfish in a desired position.

In the dissecting and study of the circulatory organs, the flattening of the skate is especially advantageous, because, one might say, it tends toward the projecting of the blood-vessels into one plane, resulting in an almost diagrammatic arrangement of them.

A practical point which will often lead to the selection of the skate, if elasmobranch material is to be used, is the fact that skates can be obtained during a large part of the year when dogfish can not. Mr. F. T. Lane, of Rockport, Massachusetts, in response to my inquiry, writes me that he can catch skates off Cape Ann at any time of the year, except during storms,

whereas dogfish can be taken in abundance only during June, July, and August, and sometimes in September. No dogfish are to be caught, he says, from the first of December to the first of May. Also in reply to inquiries of mine, Dr. F. B. Sumner, director of the laboratory of the Woods Hole station of the United States Bureau of Fisheries, states (largely, he says, on the authority of Mr. Vinal Edwards, collector for the station) that skates can be taken in the vicinity of Woods Hole at any time of year except in January, February, and March. Smooth dogfish (*Mustelus canis*) "can be taken in considerable numbers from the latter part of June till November first, being most abundant soon after their appearance late in June," while spiny dogfish (*Squalus acanthias*) are abundant only in May and early June. Neither species of dogfish can be taken in that region from the middle of November until the first of May. Similar statements as to the occurrence of dogfish and skates are made by Dr. Hugh M. Smith ('98) in his list of "The Fishes Found in the Vicinity of Woods Hole."

It appears from the foregoing statements that, so far as the New England coast is concerned, fresh skates, suitable for injection purposes, may be had throughout the greater part of the year, or possibly throughout the whole year, while fresh dogfish can be obtained during not more than five, or possibly six, months of the year. Furthermore, the dogfish are most abundant and most easily taken during the summer months when they are least wanted for laboratory purposes in schools and colleges, except as they may be preserved then and stored for future use. To be sure, dogfish may be collected and injected in the summer and preserved for use later in the year. But, to my mind, there are serious objections to giving a student his animals already injected. He should make his own injections. The injecting of the animal which he is to dissect gives the student information which he will not get so well in any other way, besides being a valuable means of developing skill in operating.

As regards the practicability of shipping fresh elasmobranch material to points distant from the coast, there is no sufficient reason why, in these days of rapid transportation, such material

cannot be had by almost any institution in the country, however remote from the coast. Fish properly packed, so as to secure coolness and freedom from too great pressure, will endure well several days' transportation, especially in the cooler seasons of the year. Skates which are intended for injecting for non-histological purposes are not injured by freezing, provided that they are not exposed to alternate freezing and thawing.

A point of minor importance is the fact that it is easier to secure good preservation of skates than of dogfish. The flattening of the skate's body facilitates the penetration of the preserving fluid to the deep organs. Furthermore, to get sexually mature animals, it is necessary to use dogfish of such large size that the preservation and storing of material for a large class become a serious problem. The same number of small sexually mature skates makes a much less bulky mass of material.

The objection that the skate is a highly specialized elasmobranch does not apply, to any great extent, to its internal anatomy. The specialization involves chiefly the general form of the body. The skeleton and muscles are the systems most affected. The remaining organs undergo relatively unimportant changes in position incident to the flattening of the body. In their essential morphological characters and relations they are closely comparable to those of the shark-like elasmobranchs. From a practical point of view, it might be said that the specialization of the skate is in the direction of adaptation for laboratory purposes.

The plan which has been followed for several years past in the laboratory of vertebrate comparative anatomy at Harvard University seems to me to be an excellent one. The student first makes a general dissection of the dogfish, exclusive of the blood vessels, then injects a fresh skate for the study of the circulatory system. By this arrangement he becomes familiar with the anatomy of the more primitive elasmobranch and studies a typical elasmobranch circulatory system under conditions far more favorable than those offered by the dogfish. At the same time, his familiarity with the dogfish enables him to detect the essential elasmobranch characteristics in the skate and to comprehend the nature of its specialization. Thus, he is

given an opportunity for observing, in an appreciative way, a remarkable case of modification of form. If the student's main work is on the skate, he should at least be given an opportunity to compare skate and dogfish far enough to make clear to him the character of the skate's departure from the more primitive conditions.

Injection Methods.

Injection of the Veins of the Skate.— What I have to say about the injection of the veins of the skate is with special reference to the best method by which this operation may be performed by students who have had little or no experience in such work. It is assumed that the injecting is to be done by means of an ordinary hand syringe.

A complete injection of the systemic veins of the skate is difficult to obtain, even under the most favorable circumstances, partly because the veins are well provided with valves which impede the flow of the injection mass in a direction the reverse of that of the blood-flow, and partly because of the presence of large thin-walled sinuses, which are likely to rupture before the injection mass can be forced into the smaller vessels and those more remote from the point of injection.

The vessels most accessible for the injection of the systemic veins are the postcardinal veins in their renal region, the venous sinus, and the lateral veins. Parker ('95) recommends introducing the injection mass at the venous sinus, the flow of the mass being directed backwards, or away from the heart. An injection directed forward into the hinder or renal part of one of the postcardinal veins has been employed with more or less success. In my opinion, however, injection by way of one of the lateral veins possesses certain advantages over either of the other methods.

The injection of the renal division of the postcardinal vein is objectionable for two reasons. In the first place, it is very difficult to dissect out the vessel. It lies under the peritoneum in a loose mass of connective tissue, and unless the operator is fairly skillful, the cannula is quite likely to be pushed into lymph

spaces or into any opening other than the proper one. In the second place, an injection mass entering by way of the renal end of the postcardinal vein passes directly to the great thin-walled cardinal sinus. No other veins can fill until the pressure in the cardinal sinus has increased sufficiently to force the mass on into the smaller and more remote spaces. This involves great danger of bursting the wall of the cardinal sinus. In practice, the danger may be lessened by exerting external pressure upon the sinus, either with the hand or by other means, so as to prevent its becoming distended to its utmost capacity.

The venous sinus is a point more favorable for injection than the postcardinal vein, so far as the filling of the vessels is concerned. If the injection mass is directed backward into one arm of the venous sinus, the initial pressure of the fluid is exerted almost directly at the ends of the several main venous trunks which, other things being equal, stand equal chances of filling. In practice, however, this method has been found objectionable when attempted by an unskillful student (and since the fish is usually the first animal injected by a student in comparative anatomy, he is quite likely to be unskillful through lack of experience). The venous sinus is not very favorably situated for the injecting operation. Great care is required lest the cannula be pushed against the delicate wall of the sinus so as to rupture it. Poor judgment in controlling the pressure on the syringe results in the bursting of the sinus, and often the thin-walled auricle is injured in the course of the operation. In the case of a preparation for demonstration or museum purposes, the cutting of the venous sinus is, in itself, objectionable.

The difficulties which are met in the injecting of the postcardinal vein or venous sinus are largely or wholly avoided by the use of the lateral vein. The lateral vein is a large vessel extending along the entire length of the side wall of the abdominal cavity and lying just beneath the peritoneum. It is conspicuous in skates which have been dead not more than a day or two, because of the blood contained in it. It is not necessary, as in the case of the renal vein, to dissect out the vessel for injection. The following method will be found practicable.

Open the abdominal cavity and note the position of the lateral

veins. Then, at a region about midway of the length of one lateral vein, and in a plane transverse to the long axis of the fish, make an incision into the muscle of the lateral wall of the abdominal cavity, carrying the incision across the lateral vein and somewhat dorsal to it (see the figure, page 377). The incision must be deep enough to admit of pressing apart the masses of muscle either side of the cut in such a way as to make easily accessible the two cut ends of the lateral vein. Immediately, then, before the loss of the blood contained in the vein shall have made it difficult to see the vessel, thrust a probe or a coarse bristle forward into the cut end of its anterior division and another backward into its posterior division. A mass which "sets" quickly may be injected without tying the cannula into the vein. For this purpose it is desirable to use a cannula of a size sufficient nearly to fill the lumen of the vein. Such a cannula, with the injection apparatus attached, may be inserted successively into the two cut ends of the vein and held firmly in place with the fingers during the process of injecting. If it is desired to tie the cannula in place, the muscle may be cut away from around the cut end of the vein so as to leave about one centimeter in length of the vessel projecting, with more or less tissue attached to it. A ligature may then be passed around this small projecting mass of tissue, including the vein, and, the cannula having been inserted, the ligature is tightened upon it.

The fluid which is injected into the anterior division of the lateral vein passes directly to the corresponding precaval (Cuvierian) sinus, whence it may pass into all the chief venous trunks of that side except as impeded by valves. It passes also, by way of the venous sinus, directly across to the opposite precaval sinus, whence it may enter the vessels of that side of the body. The cardinal and hepatic sinuses afford other, but less direct, routes for the passage of the fluid from one side to the other. In practice, this method usually results in the filling of both lateral veins and at least the proximal ends of the several brachial trunks. The mass fills also the postcardinal veins, including their posterior inosculation, the hepatic sinus and veins, the cardinal sinus, and the proximal ends of the inferior jugulars.

The precardinal veins, however, are rarely injected by this or by any other method, except that of direct injection of each precardinal from its anterior end, — an operation which is not practicable for anyone who is not familiar with the position of the vein.

The filling of the cavities of the heart may be prevented or controlled by means of ligatures tied around the heart in the regions of the sinu-auricular and auriculo-ventricular apertures.

The injection into the posterior division of the lateral vein fills the posterior connections of that vein, including the veins from the pelvic fin (see Rand and Ulrich, :05).

In brief, the advantages of injection *via* the lateral vein are as follows: (1) the lateral vein is a sufficiently large vessel and most easy of access; (2) its preparation for injection is a simple operation, requiring no nice dissection nor other delicate technique; (3) it is a relatively narrow and strong-walled vein, not easily broken by the point of a cannula, and capable of withstanding the initial pressure of the injection; (4) it conveys the mass first to the precaval sinus, whence the chief vessels may fill; (5) the weak-walled cardinal sinus is remote from the point of injection; (6) maximum filling of the systemic veins is secured by a single operation.

An injection of the renal portal system is secured easily by cutting across the tail at least four or five centimeters back of the cloacal aperture and injecting forward into the caudal vein. A taper-pointed glass cannula may be inserted carefully into the cut end of the vessel and pushed in until the end of the vein is tightly closed. During the progress of the injection the cannula must be held firmly in place. When the cannula is withdrawn, the escape of the fluid may be prevented by jamming a bit of cotton into the end of the vein.

The hepatic portal system is best injected through the mesenteric vein. To secure a full injection upon the intestine the vein must be injected backward as well as forward. Parker ('95, p. 48) injects the duodenal vein, thus getting a complete injection at one operation. This is, indeed, an advantage. But, in small skates, the greater size of the mesenteric vein, and the fact that it is so much more accessible than the duodenal vein,

make the two-way injection of it usually less difficult to the student than the single injection of the duodenal vein.

Injection Masses. — For the injection of the vessels of the skate I have used an injection mass which is merely a slight modification of well known masses, but one which may be found to possess peculiar virtues for certain special purposes. Among the injection masses suitable for purposes of gross dissection, the cold or unboiled starch mass of Pansch ('77, '81; see Whitman, '85, pp. 223–225) is doubtless best for general laboratory use, especially where, in the case of a large class, many injections must be made rapidly. It is inexpensive, easy to use, and gives results satisfactory for temporary demonstrations. It is, however, not perfectly adapted for permanent demonstrations or museum preparations, owing to the fact that it does not "set" or harden. The particles of starch remain in a discrete condition so that, in the event of any injury to the wall of an injected vessel, the mass may leak out and discolor the surrounding tissue. The starch mass is poorly adapted for the injection of the venous system of the skate because it is practically impossible to dissect the thin-walled and irregularly shaped vessels without an occasional slight injury, which gives rise to annoying leakage of the mass.

Recently I had need of a mass which should acquire, after injection, a fairly stiff consistency without becoming brittle, which should not pass through capillaries, and which should be convenient to use. Some modification of the gelatin method seemed most likely to satisfy these conditions. A gelatin mass with the coloring matter in solution, as ordinarily prepared, is designed to pass capillaries. After some unsatisfactory experiments with gelatin colored by means of pulverized carmine or insoluble Prussian blue in suspension, it was suggested by Mr. J. A. Long, who was working with me, that starch be mixed with the melted gelatin to prevent its passing into the capillary vessels. We had at hand a supply of the unboiled starch mixture in several colors. The method which we used with success at that time consisted in stirring into the melted gelatin about one fourth its volume of the thick starch mass, which always settles to the bottom of a jar containing the raw starch mixture.

Thus, the starch and the color were added both at once. In the absence of the prepared starch mixture, a similar result may be attained as follows.

Mix together some of the grocer's pulverized cornstarch and about one seventh its volume of a suitable finely powdered coloring matter (carmine, insoluble Prussian blue, chrome yellow, chrome green). Add a little cold water to the mixture and convert it into a thick paste. Into the melted gelatin stir one third or one fourth its volume of the colored starch paste. The proportions of the mixture may be varied, as occasions demand. I have found the following formula convenient: —

Melted gelatin	75 volumes.
Dry cornstarch	22 "
Dry color	3 "

For non-histological purposes it is, of course, unnecessary to use the finer grades of gelatin, such as photographic gelatin. Any ordinary culinary gelatin serves equally well, besides being always easily obtainable and less expensive. A mass of good stiffness for injection purposes is obtained by using 1 gram of dry gelatin to every 7 or 8 ccm. of water. If the vessels to be injected contain much blood, the gelatin solution must be of such strength that the mingling of the blood with it will not prevent the hardening of the mass. It is better not to inject the veins of a skate immediately after the death of the animal, for then the sinuses contain a large quantity of blood. If the fish is kept in a cool place for about two days after death, the greater part of the blood will have disappeared from the vessels. At the same time, the walls of the vessels will have relaxed so that the injection is more likely to pass into the smaller vessels. This latter consideration is of more importance with reference to the arteries than to the veins.

As regards the convenience of the starch-gelatin method, the warming of the animal, preliminary to the injection, is unnecessary. The chief difficulty, therefore, which attends the gelatin method as ordinarily used for histological purposes, is obviated. I have obtained good starch-gelatin injections of the entire circulatory system of the skate, working in a room at

ordinary temperature (20° C.) without warming the animal above the temperature of the room. The melted gelatin was slightly superheated, and the heavy brass syringe, with rubber tube and cannula, was heated by immersing in hot water to a temperature about as high as consistent with comfort in handling. By these means, the starch-gelatin was introduced into the blood-vessels at a temperature considerably above its solidifying point. The warm fluid penetrates into the smaller and remote vessels before becoming cooled sufficiently to harden, giving quite as full an injection of the finer vessels as the cold starch mass ever does.

The skates which I have injected with starch-gelatin were immersed, immediately after injection, in three or four percent formalin, which, having been freshly prepared with tap water, was considerably cooler than the room temperature. The starch-gelatin promptly solidified into a firm mass having the consistency characteristic of stiff gelatin.

The starch-gelatin method described above gives results entirely satisfactory for purposes of gross dissection. The method may be used under any conditions where the unboiled starch mass might be used, but where a mass of the consistency of gelatin is to be preferred. The advantage of the starch-gelatin as compared with the raw starch lies in the fact that the mixture "sets" and, therefore, will not escape, however much the vessels may be cut. The advantage, for the purposes mentioned, of the mixture as compared with pure gelatin is in the fact that the starch causes the mass to stop at capillaries, thus preventing danger of the injection passing from one set of vessels into another. Finally, the method is not a particularly troublesome one, since the warming of the animal itself — usually the greatest inconvenience attending the use of any warm injection mass — may be omitted. The superiority of the starch-gelatin is most marked in the injecting of a blood-system containing large thin-walled sinuses, as in the case of the venous system of the skate. Its advantages over plaster of Paris for this purpose, are obvious and it is scarcely more troublesome to use.

The injection of the arteries of the skate is a comparatively simple matter. The afferent branchial vessels may be injected

through the arterial cone, as directed by Parker ('95, p. 48). For the systemic arteries, Parker makes a single injection at the duodenal artery. But, unless one is dealing with a large skate, I have found it better to inject the anterior mesenteric artery (see Fig. 1), in spite of the fact that it is necessary to inject it backward (to secure an injection upon the intestine) as well as forward. The greater size of the anterior mesenteric artery and its accessibility, lying, as it does, along the very edge of the mesentery, are advantages which more than offset the necessity of injecting it both ways. As for details, such as the form of the cannula and the manner of inserting it, I should consider Parker's method applicable to a very large fish only. The caliber of the anterior mesenteric artery in skates of small or medium size is such as to require the use of a cannula with a slender slightly tapering tip. By Parker's method, a cannulafull of air is injected into the vessel. To avoid this, the cannula should be attached to the syringe and filled with the fluid before being inserted into the vessel.

In the accompanying figure of the skate I have indicated the points at which the injections may best be made, to secure as nearly as possible a complete injection of the blood vessels in three colors. The mesenteric vessels are conveniently reached by turning the stomach forward (so that, as the fish is viewed from the ventral side, the dorsal aspect of the stomach is seen) and pulling the digestive tube over to the animal's right side in such a way as to stretch out flat the mesentery. The figure represents the digestive tube in this position.

The several injections may well be made according to the following plan. Whatever the order, the arteries should be injected before the veins.

ARTERIES.

1. *Arterial cone* - - - - - - forward - - - blue starch-gelatin or starch.
2. *Anterior mesenteric* forward - - - red " " " "
3. " " backward - " " " " "

VEINS.

Hepatic Portal.

4. *Mesenteric* - - - - - - - - forward - - - yellow starch-gelatin or starch.
5. " - - - - - - - - backward - " " " " "

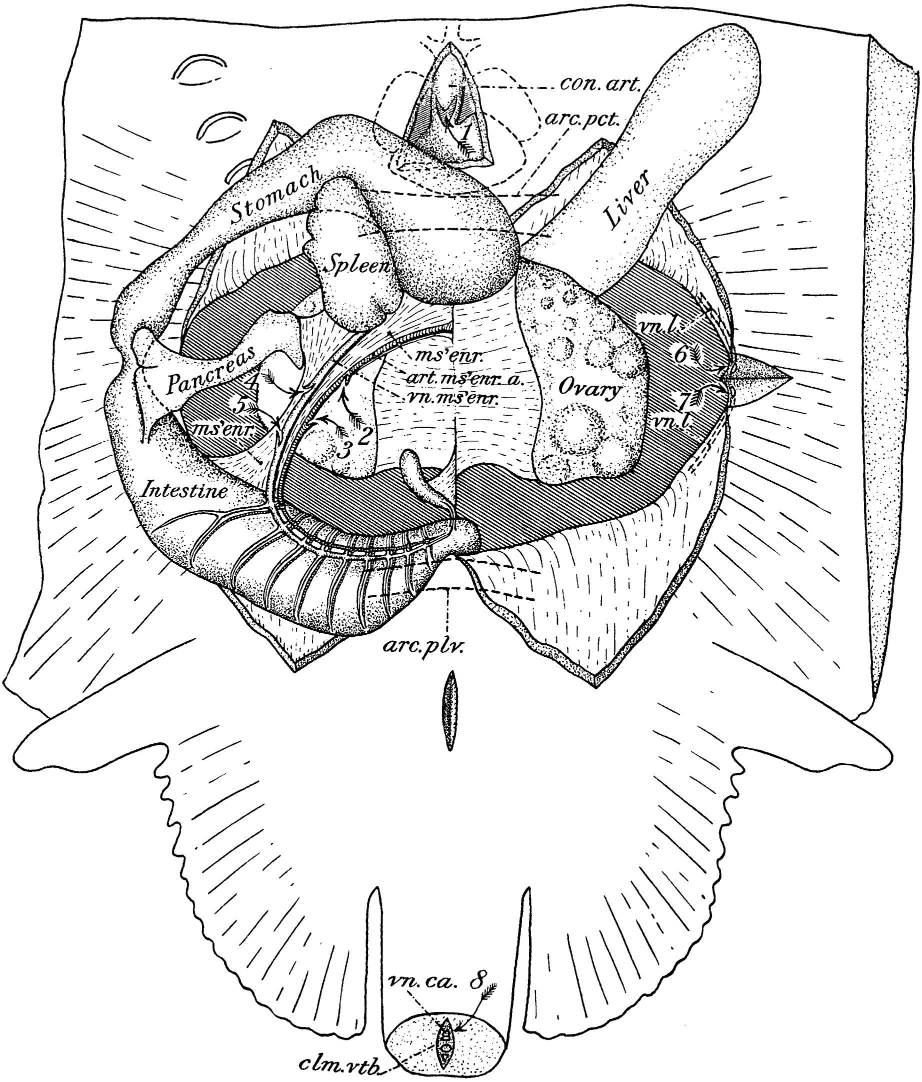

FIG. 1. — The injecting of the blood vessels of the skate. The arrows indicate the points at which the injections are to be made and the direction in which the cannula is to be inserted. The numbers attached to the arrows refer to the order of injecting given on page 376. *arc. pct.*, pectoral girdle ; *arc. plv.*, pelvic girdle ; *art. ms'enr. a.*, anterior mesenteric artery ; *clm. vtb.*, vertebral column ; *con. art.*, arterial cone ; *ms'enr.*, mesentery ; *vn. ca.*, caudal vein ; *vn. l.*, lateral vein ; *vn. ms'enr.*, mesenteric vein.

Systemic.

6. *Lateral* --------- forward --- blue starch-gelatin.

7. " ----------- backward - " " "

Renal Portal.

8. *Caudal* ----------- forward -- yellow starch-gelatin or starch.

If it is desired to use gelatin for only the systemic veins, where it is of greatest advantage, the arteries and the two portal systems may first be injected with cold starch and then the systemic veins with gelatin alone, for it is unnecessary to add starch to the gelatin to prevent its passing capillaries when all of the vessels beyond the system which is being injected are already filled.

Are so many as eight separate injections necessary? My experience has been that to decrease the number of operations is to increase their difficulty and to render the results less satisfactory. A mass injected into the systemic veins may pass through the heart into the afferent branchial vessels. Yet I have found it difficult to secure a full injection of the finer afferent vessels of the gills except by injecting directly into the ventral aorta itself. A perfectly fluid mass injected into the hepatic portal vessels or into the caudal vein may be made to fill the greater part of the venous system. But it is a great advantage in the dissection to have the hepatic portal and renal portal vessels distinguished from the systemic veins by a different color. The difference in color serves to emphasize to the mind of the student in a forcible way the nature of the relation between the portal systems and the systemic veins. The simplest way of securing the color difference is to inject each portal system separately. All the systemic arteries may be injected from the caudal artery at a single operation, but students succeed better with the two-way injection at the anterior mesenteric artery. In short, it is better to make eight easy operations than to make two or three of greater difficulty and less certainty.

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